

What is claimed is:

- 5 1. A method for the amplification of one or more target nucleic acid sequences of interest comprising:
- 10 a. contacting first and second oligonucleotide ligation probes to adjacent nucleic acid sequences of any one of said target sequences such that a 3' terminus of one of said probes is juxtaposed to a 5' terminus of the other of said probes while both the first and second probes are in contact with said target sequence,
- 15 b. ligating together said oligonucleotide probes at their respective juxtaposed termini to form a ligated target probe template;
- c. using said ligated target probe template in a strand displacement amplification reaction to form amplicons of said ligated target probe template.
- 20 2. A method of claim 1 wherein said ligation probe that is not the probe having a juxtaposed 3' terminus further having a 3' terminus that is modified such that it cannot have added thereto additional nucleotide triphosphates.
3. A method of claim 1 wherein said strand displacement amplification reaction uses a first amplification primer capable of hybridizing to all ligated target probe templates and a second amplification primer having nucleotide sequences identical to a portion of nucleotide sequences contained on said ligated target probe templates.
- 25 4. A method of claim 1 wherein no bumper primers are used in said strand displacement amplification.
5. A method of claim 1 wherein amplicons of said ligated target probe template are detected.
6. A method of claim 5 wherein detection of amplicons is by at least one of fluorescence, chemiluminescence, and electrochemiluminescence.
- 30 7. A method for the multiplex amplification of one or more target nucleic acid sequences of interest comprising:
- a. contacting first and second oligonucleotide ligation probes to adjacent

- nucleic acid sequences of any one of said target sequences such that a 3' terminus of one of said probes is juxtaposed to a 5' terminus of the other of said probes while both the first and second probes are in contact with said target sequence,
- 5      b. ligating together said oligonucleotide probes at their respective juxtaposed termini to form a ligated target probe template;
  - c. using said ligated target probe template in a strand displacement amplification reaction to form amplicons of said ligated target probe template;
  - 10     d. wherein said ligation probe that is not the probe having a juxtaposed 3' terminus further having a 3' terminus that is modified such that it cannot have added thereto additional nucleotide triphosphates;
  - e. wherein no bumper primers are used in said strand displacement amplification reaction;
  - 15     f. wherein said strand displacement amplification reaction uses a first amplification primer capable of hybridizing to said ligated target probe templates and a second amplification primer having nucleotide sequences identical to a portion of nucleotide sequences of said ligated target probe templates.
  - 20     8. A method of claim 7 wherein amplicons of said ligated target probe template are detected.
  9. A method of claim 8 wherein detection of amplicons is by at least one of fluorescence, chemiluminescence, and electrochemiluminescence.
  - 25     10. A method for the multiplex amplification of a multiplicity of target nucleic acid sequences of interest using an electronically addressable microchip comprising:
    - a. for each target nucleic acid sequences of interest;
    - b. contacting first and second oligonucleotide ligation probes to adjacent nucleic acid sequences of any one of said target sequences such that a 3' terminus of one of said probes is juxtaposed to a 5' terminus of the other of said probes while both the first and second probes are in contact with said target sequence,
  - 30

- c. ligating together said oligonucleotide probes at their respective juxtaposed termini to form a ligated target probe template;
  - d. using said ligated target probe template in a strand displacement amplification reaction to form amplicons of said ligated target probe template.
11. A method of claim 10 wherein any of said target sequence, ligated target probe template, and amplicons are electronically addressed to any of a plurality of capture sites on said microchip.
12. A method of claim 10 wherein said ligation probe that is not the probe having a juxtaposed 3' terminus further having a 3' terminus that is modified such that it cannot have added thereto additional nucleotide triphosphates.
13. A method of claim 10 wherein said strand displacement amplification reaction uses a first amplification primer capable of hybridizing to all ligated target probe templates and a second amplification primer having nucleotide sequences identical to a portion of nucleotide sequences of said ligated target probe templates.
14. A method of claim 10 wherein no bumper primers are used in said strand displacement amplification.
15. A method of claim 10 wherein amplicons of said ligated target probe template are detected.
16. A method of claim 15 wherein detection of amplicons is by at least one of fluorescence, chemiluminescence, and electrochemiluminescence.
17. A method of claim 16 wherein said amplification and detection are carried out either consecutively or simultaneously in relation to one another.
18. A method for the multiplex amplification and detection of a multiplicity of target nucleic acid sequences of interest using an electronically addressable microchip comprising:
  - a. contacting first and second oligonucleotide ligation probes to adjacent nucleic acid sequences of any one of said target sequences such that a 3' terminus of one of said probes is juxtaposed to a 5' terminus of the other of said probes while both the first and second probes are in contact with said target sequence, wherein said ligation probe that is not the probe having a

- juxtaposed 3' terminus further having a 3' terminus that is modified such that it cannot have added thereto additional nucleotide triphosphates;
- b. ligating together said oligonucleotide probes at their respective juxtaposed termini to form a ligated target probe template;
- 5 c. using said ligated target probe template in a strand displacement amplification reaction to form amplicons of said ligated target probe template, wherein no bumper primers are used in said strand displacement amplification reaction and said strand displacement amplification reaction uses a first amplification primer capable of hybridizing to all ligated target probe templates and a second amplification primer having nucleic acid sequences identical to a portion of nucleic acid sequences of said ligated target probe templates; and
- 10 d. detecting said amplicons.
- 15 19. A method of claim 18 wherein any of said target sequence, ligated target probe template, and amplicons are electronically addressed to any of a plurality of capture sites on said microchip.
- 20 20. A method of claim 18 wherein said amplification and detection are carried out either consecutively or simultaneously in relation to one another.
- 21 21. A method of claim 20 wherein detection of amplicons is by at least one of fluorescence, chemiluminescence, and electrochemiluminescence.
22. A method of claim 19 wherein said capture sites have capture probes that comprise any of: a ligation probe; a ligation probe having a 3' terminus that is modified such that it cannot have added thereto additional nucleotide triphosphates; an
- 25 amplification primer; a branched oligonucleotide comprised of a first and second amplification primer where said first amplification primer is capable of hybridizing to all ligated target probe templates and said second amplification primer has a nucleotide sequence identical to a portion of nucleotide sequences of said ligated target probe templates.
- 30 23. A method of claim 1 wherein the first and second ligation probes are incapable of being ligated together to form the ligated probe template due to a mutation in said

target sequence.

24. A method for the amplification of one or more target nucleic acid sequences of interest comprising:

- 5
- a. contacting first and second oligonucleotide ligation probes to adjacent nucleic acid sequences of any one of said target sequences such that a 3' terminus of one of said probes is juxtaposed to a 5' terminus of the other of said probes while both the first and second probes are in contact with said target sequence, wherein said first and second oligonucleotide ligation probes are initially incapable of being ligated together;
  - 10 b. rendering said first and second oligonucleotide ligation probes which are initially incapable of being ligated together, capable of being ligated together;
  - c. ligating together said oligonucleotide probes at their respective juxtaposed termini to form a ligated target probe template;
  - 15 d. using said ligated target probe template in a strand displacement amplification reaction to form amplicons of said ligated target probe template.

25. A method of claim 24 wherein said rendering comprises removing one or more terminal nucleotides from said oligonucleotide probes.

20 26. A method of claim 25 wherein one or more terminal nucleotides are removed from the 3' terminus of said second oligonucleotide ligation probe.

27. A method of claim 26 wherein one or more terminal nucleotides are removed by an endonuclease.

28. A method of claim 27 wherein said endonuclease is Endonuclease IV.

25 29. A method of claim 25 wherein one or more terminal nucleotides are removed from the 5' terminus of said first oligonucleotide ligation probe.

30. A method of claim 29 wherein one or more terminal nucleotides are removed by an exonuclease.

31. A method of claim 30 wherein said exonuclease is a DNA polymerase.

30 32. A method of claim 30 wherein one or more terminal nucleotides that are removed are non-complementary to said target sequences.

09865807-052501

09855807.052501

33. A method for the multiplex amplification of a multiplicity of target nucleic acid sequences of interest using an electronically addressable microchip comprising:
- a. for each target nucleic acid sequences of interest;
  - b. contacting first and second oligonucleotide ligation probes to adjacent nucleic acid sequences of any one of said target sequences such that a 3' terminus of one of said probes is juxtaposed to a 5' terminus of the other of said probes while both the first and second probes are in contact with said target sequence, wherein said first and second oligonucleotide ligation probes are initially incapable of being ligated together;
  - c. rendering said first and second oligonucleotide ligation probes which are initially incapable of being ligated together, capable of being ligated together;
  - d. ligating together said oligonucleotide probes at their respective juxtaposed termini to form a ligated target probe template;
  - e. using said ligated target probe template in a strand displacement amplification reaction to form amplicons of said ligated target probe template.
34. A method of claim 33 wherein said rendering comprises removing one or more terminal nucleotides from said oligonucleotide probes.
35. A method of claim 34 wherein one or more terminal nucleotides are removed from the 3' terminus of said second oligonucleotide ligation probe.
36. A method of claim 35 wherein one or more terminal nucleotides are removed by an endonuclease.
37. A method of claim 36 wherein said endonuclease is Endonuclease IV.
38. A method of claim 34 wherein one or more terminal nucleotides are removed from the 5' terminus of said first oligonucleotide ligation probe.
39. A method of claim 38 wherein one or more terminal nucleotides are removed by an exonuclease.
40. A method of claim 39 wherein said exonuclease is a DNA polymerase.
41. A method of claim 39 wherein one or more terminal nucleotides that are removed are non-complementary to said target sequences.

42. A kit for carrying out ligation-based SDA reactions for use on a bioelectronic microchip comprising:

- 5 a. one or more oligonucleotides specific for Factor V, Hemochromatosis, or a bacterium, which oligonucleotides comprise amplification primers, bumper primers, capture probes, and/or signal probes selected from the group consisting of Seq. Id. Nos. 1-62.

43. A kit according to claim 42 wherein said signal probes are labeled with a detectable label.

10

TO 5250" 20859360

